1. INTRODUCTION

The purpose of this submission is to respond to FDA's request for bioassay specifications for drug substance and drug product. Additionally, updated stability data on the drug product batches of Ovidrel® 250 mcg and Ovidrel® 500 mcg is provided.

2. TEST METHODOLOGY

2.1 Method Description for Ovidrel® Drug Product and Drug Substance

The *in vivo* biological potency of r-hCG is determined by the method described in the European Pharmacopoeia for chorionic gonadotropin. The monograph is attached in Attachment 1.

The potency of r-hCG is estimated by comparing its effect on increasing the mass of the seminal vesicles of immature rats with the same effect on a reference standard calibrated against the third international standard IS75/537.

Bioactivity is expressed in IU/mL of r-hCG solution. The specific bioactivity (IU/mg) is then calculated by dividing the bioactivity (IU/mL) by the r-hCG concentration (mcg/container). The r-hCG concentration used to calculate the specific bioactivity is determined by the SE-HPLC assay described in the analytical methods section of the NDA.

3. DRUG SUBSTANCE

3.1 BIOASSAY SPECIFICATION FOR R-HCG DRUG SUBSTANCE

The Proposed bioassay specification for r-hCG drug substance is as follows:

Table 3.1-1: Proposed Bioassay Specification for r-hCG Drug Substance

Test	Specification	Method
Specific Bioactivity	20,000-33,000 IU/mg r-hCG	Bioassay to Ph. Eur., Protein Content by SE-HPLC

3.2 JUSTIFICATION FOR THE DRUG SUBSTANCE BIOASSAY SPECIFICATION

During product development, the potency of r-hCG drug substance was estimated to be 22,000 IU / mg protein. This average specific bioactivity was generated from 13 development batches of r-hCG produced at pilot scale.

Serono now has accumulated data on 19 batches of r-hCG drug substance manufactured at full manufacturing scale, namely crude bulk production in the 300L bioreactor purified at an 800 MIU scale. The specific bioactivity calculated using these full scale batches is more representative of the true specific bioactivity of the r-hCG drug substance. Data from these batches are presented in Table 3.2-1, below.

Table 3.2-1: Specific Activity of Full Scale Batches Manufactured to Date

Batch Number	Site for USP	Site for DSP	USP Scale	DSP Scale	Specific Activity (IU/mg)
S04	LSA	IRCS	300 L	800 MIU	23,811
S05	LSA	IRCS	300 L	800 MIU	26,794
S06	LSA	IRCS	300 L	800 MIU	26,459
S07	LSA	IRCS	300 L	800 MIU	23,045
TT01	LSA	IRCS	300 L	800 MIU	25,453
TT02	LSA	LSA	300 L	800 MIU	26,187
TT03	LSA	LSA	300 L	800 MIU	28,673
BCEA9901	LSA	LSA	300 L	800 MIU	24,359
BCEA9902	LSA	LSA	300 L	800 MIU	29,427
BCEA9903	LSA	LSA	300 L	800 MIU	27,426
BCEA9904	LSA	LSA	300 L	800 MIU	28,308
BCEA9905	LSA	LSA	300 L	800 MIU	24,890
BCEA9906	LSA	LSA	300 L	800 MIU	25,008
BCEA9907	LSA	LSA	300 L	800 MIU	32,115
BCEA9908	LSA	LSA	300 L	800 MIU	28,229
BCEA9909	LSA	LSA	300 L	800 MIU	24,943
BCEA9910	LSA	LSA	300 L	800 MIU	24,763
BCEA9911	LSA	LSA	300 L	800 MIU	26,201
BCEA00501	LSA	LSA	300 L	800 MIU	25,388

Mean	26,394
Minimum	23,045
Maximum	32,115
SD	2,215
% SD	8
mean - 3SD	19,748
mean + 3SD	33,040

Based on these full scale data in Table 3.2-1, the mean specific activity is 26,400 IU per mg r-hCG. The specification for r-hCG drug substance is therefore proposed to be 20,000 - 33,000 IU per mg r-hCG which is established based on the mean specific activity plus or minus three standard deviations.

3.3 SPECIFIC ACTIVITY OF R-HCG DRUG SUBSTANCE BATCHES USED IN CLINICAL TRIALS

The batches of drug substance presented in Table 3.3-1 were used to manufacture clinical trial material during clinical development.

Table 3.3-1: Specific Activity of r-hCG Drug Substance Batches Used in Clinical Trials

Drug Substance Batches Used for Manufacture of Clinical Trial Materials	Drug Substance Specific Activity (IU/mg)	Drug Product Batch Number
T09	19,163	HCG002
S04	23,811	95CA04
S04	23,811	95CA05
S04	23,811	95CA10
S07	23,045	96CA04
S07	23,045	96CA06
S07	23,045	97CA01
S05	26,794	98CA01

All drug substance batches used to manufacture clinical trial materials were within the proposed drug substance bioassay specification, with the exception of drug substance batch T09, which was manufactured as a pilot scale batch. Batch T09 was used in the production of Ovidrel® batch HCG002 which was administered to a single patient.

4. DRUG PRODUCT

4.1 BIOASSAY SPECIFICATIONS FOR OVIDREL® DRUG PRODUCT

Proposed bioassay specifications for Ovidrel® drug product are as follows:

Table 4.1-1: Proposed Bioassay Specifications for Ovidrel® Drug Product

Test	Specification	Method
hCG potency Ovidrel 250 mcg Ovidrel 500 mcg	6000-9400 IU hCG/container (80-125% of the target fill) 12,000-19,000 IU hCG/container (80-125% of the target fill)	Bioassay to Ph. Eur, Van Hell method
Lower Fiducial Limit of error (p=0.95) of the estimated potency Ovidrel 250 mcg Ovidrel 500 mcg	NLT 4800 IU (not less than 64% of the target IU/vial) NLT 9700 IU (not less than 64% of the target IU/vial)	Bioassay to Ph. Eur, Van Hell method
Upper Fiducial Limit of error (p=0.95) of the estimated potency Ovidrel 250 mcg Ovidrel 500 mcg	NMT 11,700 IU (not more than 156% of the target IU/vial) NMT 23,700 IU (not more than 156% of the target IU/vial)	Bioassay to Ph. Eur, Van Hell method

Once sufficient commerical scale manufacturing data have been accumulated, Serono will make a proposal to delete the bioassay test from drug substance and drug product specifications and replace it with a bioidentity test for drug product.

4.2 JUSTIFICATION FOR THE OVIDREL® BIOASSAY SPECIFICATION

Ovidrel® vials are filled on the basis of mass of r-hCG as determined by SE-HPLC. Ovidrel® 250 mcg vials are filled with 285 mcg per vial to allow for mechanical losses and to ensure a delivery of 250 mcg to the patient. Ovidrel® 500 mcg vials are filled with 575 mcg per vial to allow for mechanical losses and to ensure a delivery of 500 mcg to the patient.

The mean specific activity of the bulk drug substance has been established as 26,400 IU per milligram of r-hCG as described in Section 3.2. The target fill of 285 mcg is, therefore, equivalent to 7,524 IU per vial and the target fill of 575 mcg is equivalent to 15,180 IU per vial. Bioassay specifications of 6,000 – 9,400 IU hCG / container (80 - 125% of the target fill) for Ovidrel® 250 mcg and 12,000 - 19,000 IU hCG / container (80 - 125% of the target fill) for Ovidrel® 500 mcg are proposed. The limits being applied, namely 80 - 125% of target fill, are in accordance with the current USP monograph for gonadotropins.

4.3 BIOASSAY AND SE-HPLC RESULTS FOR OVIDREL®

Bioassay results for batches of Ovidrel® 250 mcg and 500 mcg are presented in the Tables 4.3-1 and 4.3-2 below.

Table 4.3-1: Bioassay and SE-HPLC Results for 17 Batches of Ovidrel® 250 mcg

Batch Number	IU/Container (Bioassay)	Mcg/Container (SE-HPLC)	Bulk Batches Used for Manufacture
HCG002	5308	283	T09*
95CA04	6357	260	S04
95CA05	6840	293	S04
95CA10	5567	275	S04
96CA04	7217	268	S07
96CA06	7995	290	S07
96CA07	8155	293	S07
97CA01	7116	288	S07
98CA01	7753	286	S05
98CA02	7478	279	S05
98CA03	6499	268	S06
98AA01	7254	298	S06
98AA02	7470	301	S06
BZ001**	5669	282	S05
BZ002**	4912	292	S05+S06
BZ003**	5887	293	S05+S06
BZ004	8186	259	BCEA9903

* Batch T09 was manufactured at pilot scale

** Qualification Batches manufactured at IFS: Industria Farmaceutica Serono, Bari, Italy

Table 4.3-2: Bioassay and SE-HPLC Results for 7 Batches of Ovidrel® 500 mcg

Batch Number	IU/Container (Bioassay)	Mcg/Container (SE-HPLC)	Bulk Batches Used for Manufacture
97CH01	18186	583	S07
99AH01	14117	525	BCEA 9901
99AH02	15933	570	BCEA9904
99AH03	15345	568	BCEA9904
B3001*	12186	535	BCEA9905, BCEA 9906, BCEA9909
B3002*	12143	561	BCEA9903, BCEA 9909, BCEA9910
B3003*	14292	565	BCEA9904, BCEA 9908, BCEA9910

* Qualification Batches manufactured at IFS: Industria Farmaceutica Serono, Bari, Italy

Ovidrel® is a product filled and released by mass of r-hCG. A specification for bioidentity of not less than 4,000 IU / vial for Ovidrel® 250 mcg and not less than 8,000 IU / vial for Ovidrel® 500 mcg was maintained to monitor the drug product batches and was described in the Ovidrel IND 48,934. All batches manufactured during development, including those used in clinical trials and in process qualification, conformed to these development specifications.

Importantly, as a bioassay specification for Ovidrel® 250 mcg was being proposed, it became apparent that the bioassay results for the three qualification batches (BZ001, BZ002, BZ003) submitted in NDA 21-149 were below the lower limit of the proposed bioassay specification at the time of release. An investigation identified that an improper target value of 5,000 IU / vial (namely, the target value of Serono's urinary-derived hCG product, Profasi® 5,000 IU) was applied for sample preparation and calculation of results for the bioidentity test for Ovidrel® 250 mcg, which resulted in the assay being inappropriately centered. This was corrected in late 1999. As evidenced by the stability data, all batches are within the proposed specification. Updated stability information for the 3 qualification batches of Ovidrel® 250 mcg and the 3 qualification batches of Ovidrel® 500 mcg can be found in Attachments 2 and 3, respectively.

Table 4.3-3: Specific Activity of Ovidrel® Batches Used in Clinical Trials

Batch No.	Bioassay Result IU/vial	mcg/container	Number of Patients		
			Study 7648	Study 7927	Study 8209
HCG002	5,308	283	1	0	0
95CA04	6,357	260	64	158	3
95CA05	6,840	293	32	18	13
95CA10	5,567	275	0	7	12
96CA04	7,217	268	0	0	8
96CA06	7,995	290	0	0	27
97CA01	7,116	288	0	0	8
98CA01	7,753	286	0	0	4

During clinical studies, a majority of patients (95%) received Ovidrel® drug product that conformed to the proposed specification. Five percent of patients received product manufactured from one of two batches [batch HCG002 (5,308 IU) or batch 95CA10 (5,567 IU)] with an activity marginally below the proposed lower limit of the specification (6,000 IU), as illustrated in Table 4.3-3.

Batch HCG002, was manufactured from pilot scale drug substance material that had a specific activity (19,163 IU/mg) marginally below the proposed specification (20,000 IU/mg). However all remaining clinical trial material was manufactured from drug substance batches with a specific activity meeting the proposed specification.

In conclusion, stability results for the three qualification lots for Ovidrel® 250 mcg tested to date demonstrate average potency values (BZ001 = 7079 IU/vial, BZ002 = 6501 IU/vial and B/003 = 7506 IU/vial) which are within the proposed bioassay specifications confirming the acceptability of these batches.

5. STABILITY

The bioassay specifications contained in the drug product stability protocols were applied during development. Therefore, the protocol will be revised to include the updated proposed specification.

Updated stability information for the 3 qualification batches of Ovidrel® 250 mcg (Batches BZ001, BZ002, BZ003) as well as a subsequent production batch (BZ004), and the 3 qualification batches of Ovidrel® 500 mcg (B3001, B3002, B3003) can be found in Appendix 2 and 3, respectively.

Attachment 1

Ph. Eur. Bioassay for Chorionic Gonadotropin

Acetic acid. If the product is the acetate, it complies with the following additional requirement. Not more than 7.5 per cent *m/m*, determined by gas chromatography *(2.2.28)*, using *dioxan R* as internal standard.

Test solution (a). Dissolve 20.0 mg of the substance to be examined in 1.0 ml of *water R*.

Test solution (b). Dissolve 20.0 mg of the substance to be examined in 1.0 ml of *water R* and add 10 µl of *dioxan R*.

Reference solution. Dissolve 1.0 mg of *glacial acetic acid R* in 1.0 ml of *water R* and add 10 µl of *dioxan R*.

The chromatographic procedure may be carried out using:

- a glass column 2 m long and 2 mm in internal diameter packed with *ethylvinylbenzene-divinylbenzene copolymer R* (125 µm to 180 µm),
- *nitrogen for chromatography R* as the carrier gas,
- a flame-ionisation detector,

maintaining the temperature of the column at 150 °C.

Chlorides *(2.4.4).* If the product is the hydrochloride, it complies with the following additional requirement. Dissolve 0.83 mg in 15 ml of *water R*. The solution complies with the limit test for chlorides (6 per cent).

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Test solution. Dissolve 1.0 mg of the substance to be examined in a mixture of 15 volumes of *acetonitrile R* and 85 volumes of phosphoric acid (10 g/l H_3PO_4) adjusted to pH 3.0 with *triethylamine R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution. Dissolve 1.0 mg of *gonadorelin CRS* in a mixture of 15 volumes of *acetonitrile R* and 85 volumes of phosphoric acid (10 g/l H_3PO_4) adjusted to pH 3.0 with *triethylamine R* and dilute to 10.0 ml with the same mixture of solvents.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),
- as mobile phase at a flow rate of 1.0 ml per minute a mixture of 75 volumes of phosphoric acid (10 g/l H_3PO_4) adjusted to pH 3.0 with *triethylamine R* and 25 volumes of *acetonitrile R*; adjust the proportions of the mixture so that the retention time of gonadorelin is about 10 min,
- as detector a spectrophotometer set at 220 nm.

Inject separately 20 µl of each solution.

Calculate the content of the peptide $C_xH_yN_nO_n$ using the declared content of $C_{55}H_{75}N_{17}O_{13}$ in *gonadorelin CRS*. The assay is not valid unless the number of theoretical plates is at least 20 000 per metre.

STORAGE

Store in an airtight container, protected from light at a temperature of 2 °C to 8 °C.

LABELLING

The label states:

- the mass of peptide in the container,
- the storage conditions,
- where applicable, that the contents are the acetate,
- where applicable, that the contents are the hydrochloride.

1997:0498

GONADOTROPHIN, CHORIONIC

Gonadotropinum chorionicum

DEFINITION

Chorionic gonadotrophin is a dry preparation of placental glycoproteins which have luteinising activity. The potency is not less than 2500 I.U. per milligram.

PRODUCTION

Chorionic gonadotrophin is extracted from the urine of pregnant women using a suitable fractionation procedure. It is either dried under reduced pressure or freeze-dried. It is prepared in conditions designed to minimise or eliminate microbial and viral contamination. The manufacturing process must have been shown to reduce any viral contamination such as hepatitis virus or HIV by appropriate validated methods.

CHARACTERS

A white to yellowish-white, amorphous powder, soluble in water.

IDENTIFICATION

When administered to immature rats as prescribed in the assay, it causes an increase in the mass of the seminal vesicles and of the prostate gland.

TESTS

Water. Not more than 5 per cent *m/m*, determined by gas chromatography *(2.2.28)*, using *anhydrous methanol R* as the internal standard.

Use dry glassware (which may be silicone-treated).

Internal standard solution. Dilute 15 µl of *anhydrous methanol R* to 100 ml with *2-propanol RI*.

Test solution (a). Dissolve 4 mg of the substance to be examined in 0.5 ml of *2-propanol RI*.

Test solution (b). Dissolve 4 mg of the substance to be examined in 0.5 ml of the internal standard solution.

Reference solution. Add 10 µl of *water R* to 50 ml of the internal standard solution.

The chromatographic procedure may be carried out using:

- a stainless steel column 1 m long and 2 mm in internal diameter packed with *styrene-divinylbenzene copolymer R* (180 µm to 250 µm),
- *helium for chromatography R* as the carrier gas,
- a thermal-conductivity detector.

Maintain the temperature of the column at 120 °C and that of the detector at 150 °C. Inject the chosen volumes of each solution. Calculate the content of water assuming its density (2.2.5) at 20 °C to be 0.9972 g per millilitre and taking into account any water detectable in the internal standard solution.

Sterility (2.6.1). If intended for use in the manufacture of parenteral dosage forms without a further appropriate sterilisation procedure, it complies with the test for sterility.

Pyrogens (2.6.8). If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a quantity equivalent to 300 I.U. dissolved in not more than 1 ml of a sterile, pyrogen-free 9 g/l solution of sodium chloride.

ASSAY

The potency of chorionic gonadotrophin is estimated by comparing under given conditions its effect of increasing the mass of the seminal vesicles (or the prostate gland) of immature rats with the same effect of the International Standard of chorionic gonadotrophin or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Standard, which consists of a mixture of a freeze-dried extract of chorionic gonadotrophin from the urine of pregnant women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Use immature male rats of the same strain, 19 to 28 days old, differing in age by not more than 3 days and having body masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of at least five animals. If sets of six litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose three doses of the reference preparation and three doses of the preparation to be examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 4 I.U., 8 I.U. and 16 I.U. may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate albumin buffered saline pH 7.2 R* such that the daily dose is administered in a volume of about 0.5 ml. Add a suitable antimicrobial preservative such as 4 g/l of phenol or 0.02 g/l of thiomersal. Store the solutions at 5 ± 3 °C.

Inject subcutaneously into each rat the daily dose allocated to its group, on four consecutive days at the same time each day. On the fifth day, about 24 h after the last injection, kill the rats and remove the seminal vesicles. Remove any extraneous fluid and tissue and weigh the vesicles immediately. Calculate the results by the usual statistical methods, using the mass of the vesicles as the response. (The precision of the assay may be improved by a suitable correction of the organ mass with reference to the body mass of the animal from which it was taken; an analysis of covariance may be used).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error (P = 0.95) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

Store in an airtight, tamper-proof container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the number of International Units per container,
- the potency in International Units per milligram,
- where applicable, that the substance is sterile.

1997:0719

GONADOTROPHIN, EQUINE SERUM FOR VETERINARY USE

Gonadotropinum sericum equinum ad usum veterinarium

DEFINITION

Equine serum gonadotrophin for veterinary use is a dry preparation of a glycoprotein fraction obtained from the serum or plasma of pregnant mares. It has follicle-stimulating and luteinising activities. It may be prepared by precipitation with alco-